



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Phosphorus 30 CH to control Varroa population in Apis mellifera colonies

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Phosphorus 30 CH to control Varroa population in Apis mellifera colonies / Moscatelli, Francesca; Pietropaoli, Marco; Brocherel, Giuseppina; Martini, Andrea; Formato, Giovanni. - In: EUROPEAN JOURNAL OF INTEGRATIVE MEDICINE. - ISSN 1876-3820. - STAMPA. - 8:(2016), pp. 861-864.

Availability:

This version is available at: 2158/1072741 since: 2017-06-27T11:13:47Z

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)



Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/eujim



Research paper

Phosphorus 30 CH to control *Varroa* population in *Apis mellifera* colonies

Francesca Moscatelli^a, Marco Pietropaoli^a, Giuseppina Brocherel^a, Andrea Martini^b,
Giovanni Formato^{a,*}

^a Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Via Appia Nuova 1411, 00178 Rome, Italy

^b Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Università di Firenze, Piazzale delle Cascine 18, Florence, Italy

ARTICLE INFO

Article history:

Received 27 November 2015

Received in revised form 12 June 2016

Accepted 13 June 2016

Available online xxx

Keywords:

Phosphorus 30 CH

Varroa destructor

Homeopathy

Honeybee

ABSTRACT

Introduction: *Varroa destructor* is considered to be the main cause of European honeybee (*Apis mellifera* ssp. Linnaeus 1758) colony losses. The use of homeopathic products in veterinary practices has consistently increased in the last 50 years, but limited data are available on the application of homeopathic treatments to honeybees.

The aims of this study were to investigate the acaricide efficacy and tolerability for honeybees treated for 35 days with the homeopathic product Phosphorus 30 CH.

Methods: The clinical trial was carried out during the summer of 2012 in Central Italy. Twenty-four honeybee colonies were evenly divided into two different groups: one treated with Phosphorus 30 CH (12 colonies) and one left untreated (12 colonies). The mite mortality rate of the remedy was evaluated by counting the number of fallen mites on the sticky boards placed on the bottom tray of the hives every 3 days. Oxalic acid administration in an absence of brood was used to estimate the number of surviving mites. To assess the honeybee tolerability to the treatment, immediately before and after the Phosphorus 30 CH administration, an evaluation of the adult honeybee population was performed.

Results: The results revealed that no efficacy differences (p -value = 0.079, U = 23; EV = 45; Variance (U) = 150), nor differences in hive strengths (p -value = 0.118; U = 25.5; EV = 45; Variance (U) = 147.76) were observed in the treated group compared to the untreated group.

Conclusion: Our results are consistent with other studies conducted using homeopathic remedies to control varroa mites in *Apis mellifera* colonies. Further studies are needed to compare our data with different treatment durations, different administration methods and potency of the remedy.

© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

Homeopathy was developed in 1796 by Samuel Hahnemann. It is a system of alternative medicine based on the principle that *like cures like*, according to which, a substance causing the symptoms of a disease is able to cure the disease [9]. The use of homeopathic products in veterinary practices has consistently increased in the last 50 years [10,17].

Today, *Varroa destructor* (*V. destructor*), considered the main parasite of the European honeybee (*Apis mellifera* Linnaeus 1758), has spread to beehives worldwide, with the exception of Australia, and is a major threat for apiculture [21,8].

V. destructor is considered a crucial factor in the decreasing numbers of honeybee colonies and beekeepers [3]. Increased tolerance to different synthetic active molecules has been reported

in strains of *V. destructor* that are resistant to fluvalinate, flumethrin and amitraz [1,5]. The use of natural treatments instead of hard chemicals and synthetic molecules has the advantage of limiting the drug resistance within the pathogens of farm animals [25,18,8], avoiding the environmental contamination and persistence of active compounds residues or their metabolites in the honeybee products. Moreover, homeopathic products can also decrease the cost of procedures and reduce health hazards to humans during treatment of animals [13,24]. Finally, the application of the homeopathic remedies should encourage beekeepers to adopt good farming methods [14], simultaneously enhancing the honeybees' welfare and immune systems [16].

Few data are available regarding the application of homeopathic treatments for honeybee diseases. In their preliminary studies, Sassoli et al. [23] tested the efficacy of *Calcarea Sulphurica* in improving the resistance to *Varroa* mites in beehives. Lotti and Martini [15] tested the efficiency of *Calcarea sulphurica* 200 CH and ApiBioxal[®] to improve the resistance to varroosis of honeybee

* Corresponding author.

E-mail address: giovanni.formato@izslt.it (G. Formato).

families treated in an absence of brood. With the same purpose, Persano and Marinelli [19] tested three homeopathic products: *Eureka*, *Apeas plus*, and *Apedin Vapor*. Other studies have been conducted by Ruiz Espinoza and Guerrero Salinas [22] that have attempted to build up an experimental protocol for the control of *V. destructor*, including the frequency of application and the potency (202 CH) of *Sulphur* and *Varroa Nosode*. To control *Varroa* mites in 2005, Flores [7] applied *Equisetum* spp. 60 CH with or without succussion directly to the feed administered to the honeybees.

Due to the lack of information on the application of homeopathy to honeybee diseases, this study aimed to investigate the tolerability of the *Phosphorus 30 CH* treatments on honeybees and its efficacy in improving resistance against *V. destructor*.

2. Methods

The field trial aimed to verify efficacy of *Phosphorus 30 CH* in improving resistance of honeybees to *V. destructor* after 35 days of treatment in presence of brood and to verify the honeybees' tolerability of this treatment. The clinical trial was carried out from June 2012 to the end of July 2012 in Rome province (Central Italy) on 24 honeybee colonies, located in the same apiary (41°43'28.1"N 12°42'57.1"E), housed in 10-frame Dadant-Blatt bee hives and free of any other symptomatic disease.

The 24 hives were divided into two homogeneous groups: (1) 12 colonies treated with *Phosphorus 30 CH* ("Phosphorus" group) and (2) 12 colonies left untreated ("Control" group). To evenly distribute the colonies in the two groups according to the *Varroa* population in each colony and the hive strength, the initial *Varroa* infestation level of the hives was evaluated by the natural mite fall counts recorded for two weeks before starting the trials [2], while the adult honeybee population was estimated by the Liebefeld estimation method [11].

The protocol is shown in Fig. 1 for the "Phosphorous" group and Fig. 2 for the "Control" group.

The *Phosphorus 30 CH* adopted remedy was selected according to scientific literature on symptoms transferred from humans to *Apis mellifera* ethological characteristics [4,12]. We considered as conceivable characteristics for honeybees: irritability; sensitivity (as reported by Kent [12]: "the patient phosphorus is very sensitive to all external sensations"); busy; perseverance; apprehensive during the "storm". One gram of *Phosphorus 30 CH* (Boiron laboratory Ltd, 20124 Milan, Italy) was diluted in 10 ml of water and the mixture was dissolved in a 1:1 ratio of sucrose, avoiding the use of metal tools to mix the product. Once a week, 1 ml of the *Phosphorus 30 CH* sugar solution was poured on a lump of sucrose positioned on the frames in the brood box (Fig. 3), for a total of 5 doses to cover 35 days of treatment.

To assess the honeybees' tolerability to the treatment, immediately before the first *Phosphorus 30 CH* administration (day 0) and one week after the 5th treatment (day 35), the adult

honeybee population was evaluated using the Liebefeld estimation method [11]. Finally, the honeybee queens' vitality was recorded weekly during the 35 days of the treatment.

The experimental protocol (Figs. 1 and 2) in the apiary for testing the efficacy of *Phosphorus 30 CH* against *V. destructor* was conducted according to the EU guidelines [6]. For the field trial period, the mite fall was recorded every 3 days through the use of sticky boards put on the bottom tray of the hives.

To estimate the number of surviving mites after a period of 35 days of treatment administration, we counted *Varroa* fall produced by the administration of a single dose of trickled oxalic acid solution (Apibioxal, Chemicals Laif s.r.l.) after 24 days of queen caging with VAR-CONTROL[®] cages (Api-Mo.Bru, Campodoro, Padova, Italy – <http://www.apimobru.com/en/ppe/ppe.htm>) and considering a treatment efficacy of 90% [20].

The percentage of acaricide efficacy (AE) in each hive was evaluated using the formula:

$$AE = \frac{V_{\text{Phosph 30 CH}}}{V_{\text{Tot (Phosph 30 CH + Oxalic acid)}}} * 90$$

where V_{Phosph} is the total number of mites eliminated with the treatment with *Phosphorus 30 CH*, and $V_{\text{Tot(Phosph30CH+Oxalicacid)}}$ represents the total number of mites removed by the *Phosphorus 30 CH* treatment, plus the oxalic acid treatments.

The mean acaricide efficacy in the two groups (\overline{AE}) was evaluated using the formula:

$$\overline{AE} = \frac{\sum V_{\text{Phosph 30 CH}}}{\sum V_{\text{Tot (Phosph 30 CH + Oxalic acid)}}} * 90$$

Statistical analysis was performed to compare the efficacy of the treatment and the strength of the hives between the two experimental groups. The analysis only included colonies that had a level of infestation between 300 and 3,000 mites per colony, as indicated in the guideline on veterinary medicinal products controlling *V. destructor* parasitosis in bees [6]. Considering that the data do not come from a normally distributed population, the comparisons between groups were carried out with a nonparametric Mann-Whitney test for independent samples. The significance level was set to 0.05. All the statistical analyses were performed using XLSTAT software.

3. Results

The two groups, placed in the same site before starting the trial, presented homogeneous results, having a similar infestation level (p -value=0.540; U =37.0; EV =45; Variance (U)=150.00) and strength (p -value=0.901; U =47.0; EV =45; Variance (U)=145.395).

The acaricide efficacy observed in the "Phosphorus" group was 46.1%, while the acaricide efficacy observed in the "control" group

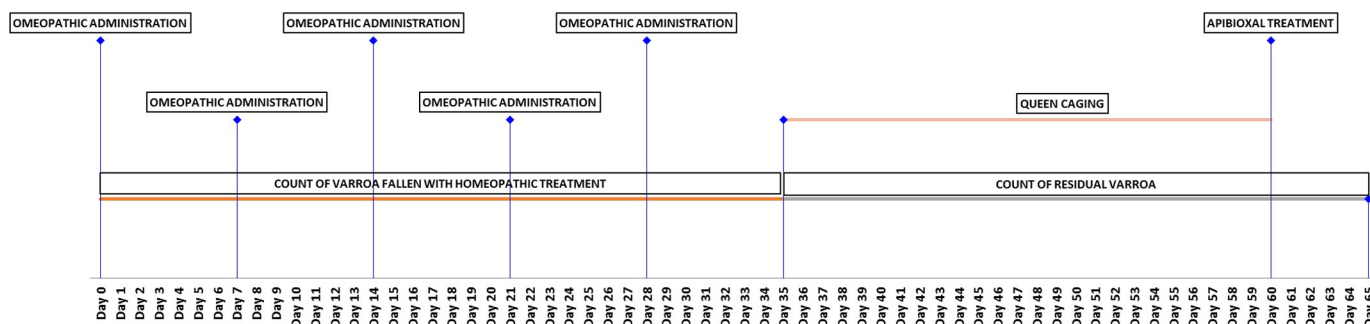


Fig. 1. Scheme of the protocol adopted for the "Phosphorus" group.

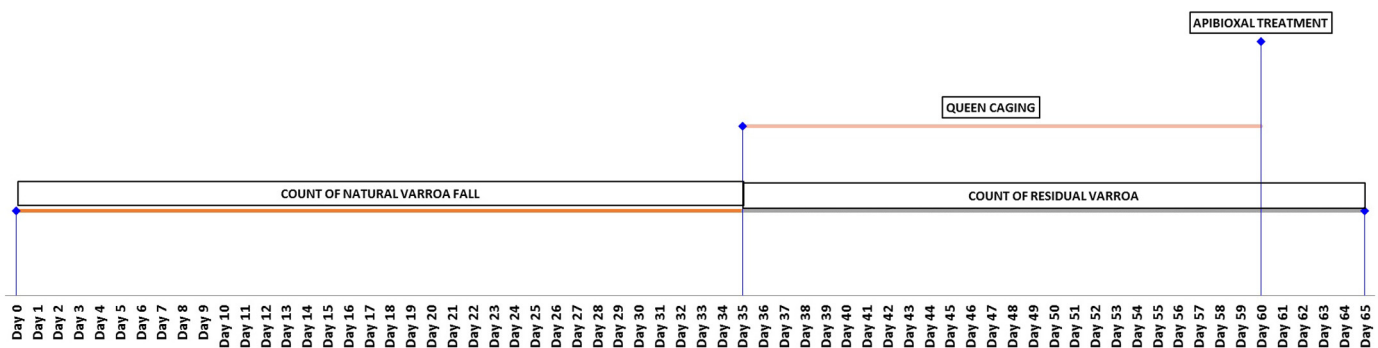


Fig. 2. Scheme of the protocol adopted for the "Control" group.



Fig. 3. Remedy administration to the honey bees by a lump of sugar.

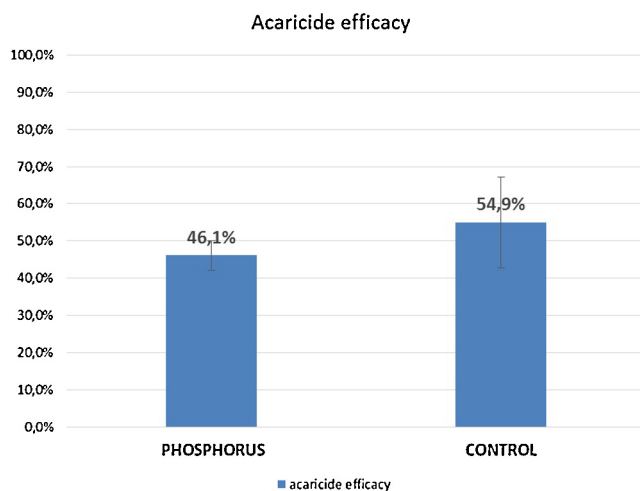


Fig. 4. Acaricide efficacy.

was 54.9% (Fig. 4). The standard deviation (SD) was 4.0% in the *Phosphorus* group and 12.1% in the Control group. No statistical differences were observed (p -value = 0.079, $U = 23$; $EV = 45$; Variance (U) = 150) between the two groups. Considering the toxicity of the treatment with *Phosphorus* 30 CH on the adult honeybees, in the "Phosphorus" group, after the treatment it was possible to observe an increase in the adult honeybee population of 19.74%, with an SD of 31.2%, comparing to an increase in the "control" group of 34.23% with an SD of 42.6% (Table 1). Even in this case, no statistical differences were observed between the two groups (p -value = 0.118; $U = 25.5$; $EV = 45$; Variance (U) = 147.76). After the treatment (day 35), the deaths of 3 queens in the "Phosphorus" group and 1 in the control group were observed.

4. Discussion

In our evaluation of the tolerability of the treatment, we did not observe any toxic effect on the adult honeybee populations, confirming the harmlessness of the homeopathic remedies.

However, we observed unsatisfactory results with *Phosphorus* 30 CH in the control of the *Varroa* population, perhaps due to the methods of use we adopted: duration of the treatment (35 days), method of administration (the lump of sugar once a week) and potency of the remedy (30 CH). Many factors that may interfere with the evaluation of the efficacy of homeopathic treatments must be considered, including the remedy, the potency of the remedy, the method of administration, the climatic conditions, the duration of the treatment and the honeybee subspecies [19,22,23]. Further studies modifying one or more of the factors mentioned above are needed to verify conceivable improvement in the control of the parasite.

It should also be taken into consideration that the homeopathic remedy must not have any acaricide effects, but aims to create a balance between host and parasite with infestation decrease.

Sassoli et al. [23] and Lotti and Martini [15] treated the honeybee families with *Calcarea sulphurica*, associating the treatment with Api-Bioxal®, and observed that the acaricide efficacy did not differ between the treated and untreated group. Persano and Marinelli [19] showed that after the treatments with the homeopathic remedies *Eureka* and *Apedin Vapor* (water, ethanol 19%, lactose and plant extracts: *Echinacea angustifolia*, *Thuya occidentalis*, *Spiraea ulmaria* and *Oxalis acetosella*)

Table 1

Evaluation of the strength of the honeybee hives in the two experimental groups before and after the *Phosphorus* 30 CH treatment.

	Pre-treatment (honeybees)	Post-treatment (honeybees)	Increase
"Phosphorus" group	51082.5	59190.59	54008.09 (+19.74%)
"Control" group	55136.44	70542.21	15405.77 (+34.23%)

administered by dripping and *Apeas plus* (administered by spraying), the number of mites in the honeybee colonies persisted at high levels.

Ruiz Espinoza and Guerrero Salinas [22] tried to control *V. destructor* by the application of *Sulphur* 202 CH to the honeybees through impregnated sugar globules and noted no effective control of the mites. Finally, in 2005, Flores [7] found that *Equisetum* spp. 60 CH showed an efficacy of 57% in controlling varroa mites by direct administration to the feed of the honeybees. Our results are consistent with those presented in the above-mentioned studies.

References

- [1] J. Baxter, F. Eischen, J. Pettis, W.T. Wilson, H. Shimanuki, Detection of fluvalinate resistant *V. mites* in U.S. honeybees, *Am. Bee J.* 138 (4) (1998) 291.
- [2] M.R. Branco, N.A.C. Kidd, R.S. Pickerd, A comparative evaluation of sampling methods for *Varroa destructor* (Acari: Varroidae) population estimation, *Apidologie* 37 (2006) 452–461.
- [3] P. de la Rua, R. Jaffe, R. Dall'Olio, I. Munoz, J. Serrano, Biodiversity, conservation and current threats to European honey bees, *Apidologie* 40 (2009) 263–284.
- [4] H. Duprat, *Materia Medica Omeopatica*, Fratelli Palombi Editori, Roma, 1983.
- [5] P.J. Elzen, J.R. Baxter, M. Spivak, W.T. Wilson, Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos, *Apidologie* 31 (2000) 437–441.
- [6] European Medicines Agency, Committee for Medicinal Products for Veterinary Use Guideline on Veterinary Medicinal Products Controlling *Varroa destructor* Parasitosis in Bees, (2008) EMA/CVMP/EWP/459883/2008. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/11/WC500099137.pdf. (accessed 23.06.14).
- [7] S. Flores, Evaluación De La Dinamización Homeopática De Cola De Caballo (*Equisetum arvense*) Para El Control De ácaro (*Varroa jacobsoni*) En Abejas (*Apis mellifera*), Departamento de Agroecología, UACH, Chapingo, México, 2005, pp. 56.
- [8] V. Goswami, M.S. Khan, Management of varroa mite, *Varroa destructor* by essential oil and formic acid in *Apis mellifera* Linn. colonies, *J. Nat. Prod.* 6 (2013) 206–210.
- [9] S. Hahnemann, J. Kunzli, A. Naude, P. Pendleton, *Organon of Medicine*, Gollancz, London, 1983.
- [10] K.E. Hammarberg, Animal welfare in relation to standards in organic farming, *Acta Vet. Scand. Suppl.* 95 (2001) 17–26.
- [11] A. Imdorf, L. Gerig, Course in determination of colony strength, Swiss Federal Dairy Research Institute, Liebefeld CH-3003 Bern Switzerland (after L. Gerig, 1983. Lehrgang zur Erfassung der Volksstärke). Schweiz Bienen-Zeitung 106: (2001) 199–204.
- [12] J.T. Kent, *Materia medica omeopatica – Le lezioni classiche di J.T Kent sui 179 rimedi essenziali dell' omeopatia* (2003).
- [13] A. Kijlstra, I.A.J. Eijck, Animal health in organic livestock production systems: a review, *NJAS – Wageningen J. Life Sci.* 54 (1) (2006) 77–94.
- [14] K. Johnoson, Bees?: more questions than answers, *Homeopathy in practice*, (2009) p. 60–63.
- [15] C. Lotti, A. Martini, *Calcarea sulphurica* protects bees against varroasis, *Homeopathy in practice*, Summer (2013).
- [16] A. Martini, V. Ferrante, S. Barbieri, Edizione Italiana del testo a cura di, *Salute e benessere animale in agricoltura biologica*, Edagricole, Bologna, IT, in: S. Vaarst, V. Lund, W. Lockeretz (Eds.), *Animal Health and Welfare in Organic Agriculture*, CABI Publishing, 2006, pp. 266.
- [17] R. Mathie, L. Hansen, M. Elliott, J. Hoare, Outcomes from homeopathic prescribing in veterinary practice: a prospective, research-targeted, pilot study, *Homeopathy* 96 (1) (2007) 27–34.
- [18] M.B. Molento, Parasite control in the age of drug resistance and changing agricultural practices, *Vet. Parasitol.* 163 (3) (2009) 229–234.
- [19] L. Persano Oddo, E. Marinelli, Impiego di prodotti omeopatici nella lotta contro *Varroa destructor* Anderson & Trueman. In Atti XIX Congresso Nazionale di Entomologia, Catania, Italia, (2002) 10–15 Giugno, 2002. Ministero delle Politiche Agricole e Forestali Istituto Sperimentale per la Zoologia Agraria, Sezione di Apicoltura, Roma.
- [20] M. Perugini, A. Giacomelli, M. Milito, M. Pietropaoli, M. Pizzariello, F. Scholl, G. Formato, L'engagement des reines: un premier bilan, *Proc. Journée Scientifique Apicole* (2013) 68–73.
- [21] P. Rosenkranz, P. Aumeier, B. Ziegelmann, Biology and control of *Varroa destructor*, *J. Invertebr. Pathol.* 103 (Suppl. 1) (2010) S96–S119.
- [22] F.J. Ruiz Espinoza, J.I. Guerrero Salinas, Resultado de investigación de Homeopatía en abejas (*Apis mellifera* m. L.). Fase I Control homeopático de ácaros (*Varroa destructor* Oud.) en abejas, *La homeopatía de México* 74 (637) (2005) 112–120.
- [23] C. Sassoli, C. Lotti, A. Martini, D. Pradella, G. Ragona, G. Brajon, Valutazione di trattamenti omeopatici per il rafforzamento della resistenza alle più comuni patologie di famiglie di api allevate con metodo biologico, *Quaderno SOZOOALP* 7 (2012) 143–152. http://www.sozooalp.it/fileadmin/superuser/quaderni/quaderno_7/Quaderno_SZA7_Completo.pdf.
- [24] S.M. Tabassam, Z. Iqbal, A. Jabbar, Z. Sindhu, A.I. Chattha, Efficacy of crude neem seed kernel extracts against natural infestation of *Sarcoptes scabiei* var. ovis, *J. Ethnopharmacol.* 115 (2) (2008) 284–287.
- [25] H.M. Thompson, M.A. Brown, R.F. Ball, M.H. Bew, First report of *Varroa destructor* resistance to pyrethroids in the UK, *Apidologie* 33 (4) (2002) 357–366.